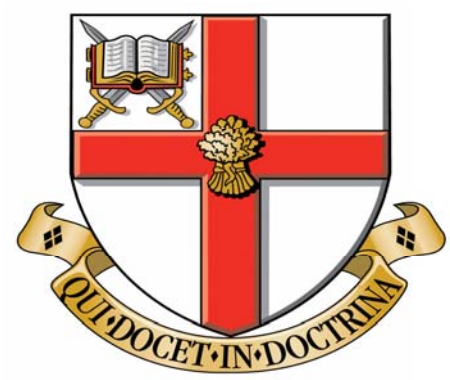

University of Chester

Department of Clinical Sciences and Nutrition

MSc Exercise and Nutrition Science

Module Title: Research Project

Module Code: XN7523

September 2014

Liam Holmes

1224529

Assessment Number: J12364

Word count: Review Paper - 5283

Word count: Research article - 4298

Table of contents

Research Article

Abstract

Introduction

Saliva sampling

I. Salivary immunoglobin A
II. Cortisol

Blood sampling

I. Creatine Kinase
II. Urea

Method

I. Experimental approach to the problem
II. Subjects
III. Sampling procedures
IV. Statistical analysis

Results

Discussion

I. SIgA
II. Cortisol
III. Urea
IV. Creatine kinase

Limitations to the study

Conclusion

References

Ethical approval documentation

Department of Clinical Sciences and Nutrition

MSc Exercise and Nutrition Science

Module Code: XN7523

September 2014

Review Paper -

Monitoring Physiological Biomarkers in Soccer

Assessment Number J12364

Word count: 5283

Abstract

Saliva and blood sampling has been increasingly used as a diagnostic tool for the assessment of physiological biomarkers in elite sport. In elite level soccer, considerable physiological and psychological stress can be experienced throughout the season. The consequence of having periods where players are in a sub optimal physiological state could lead to a reduction in performance, impaired immunity and increased risk of infection. Monitoring the variations and relationships of salivary and blood biomarkers in response to exercise and specifically elite soccer can provide detailed information on the physiological status of the players. This review will focus on the responses of salivary immunoglobin A (sIgA), cortisol, urea, and creatine kinase (CK) to exercise and elite soccer.

Introduction

Modern day soccer is classed as a high intensity intermittent activity (Dellal et al. 2011). An outfield player can cover distances ranging from 10-13km (Bradley, Sheldon, Wooster, Olsen, Boanas & Krustrup, 2009) whilst performing 1500 - 3000m of high intensity activity during a match (movement >18km/h) (Bradley, Sheldon, Wooster, Olsen, Boanas & Krustrup, 2009). The high level of physical activity coupled with the limited opportunity to schedule sufficient recovery may influence body homeostasis (Heisterberg, Fahrenkrug, Krustrup, Storskov, Kjær & Andersen, 2013). During a premier league season there are considerable physical demands as a result of training alongside the ever-increasing fixture congestion (Bloomfield, Polman & O'Donoghue, 2007). As a result there is huge emphasis on optimising recovery

from both training and matches (Mohr et al. 2010). The measurement of physiological biomarkers in whole saliva and blood can offer a valuable tool for assessing the hormonal, immunological and endocrinological status associated with exercise and training (Ascensão, Rebelo, Oliveira, Marques, Pereira & Magalhães, 2008; Papacosta & Nassis, 2011).

Failure to recover adequately from competitive games and training has been associated with the occurrence of immune suppression, a reduction in anaerobic performance and reduced neuromuscular output in soccer players (Da Silva, Papoti, Santhiago, Pauli and Gobatto, 2011; Malm, Ekblom & Ekblom, 2004). Acute bouts of strenuous exercise can compromise various aspects of immune function by revealing an ‘open window’ of increased susceptibility to infection (Gleeson & Walsh, 2012). This occurs due a temporary fall in the circulating natural killer (NK) cell count alongside increases in leukocytes, neutrophils and pro inflammatory cytokines (Walsh, Laing, Oliver, Montague, Walters & Bilzon, 2004). In one study, the reduction in NK cell count was evident for seven days following exercise (Nieman et al. 1990). However, the majority of the research concludes that the depression of circulating NK cell levels is only evident for only a few hours, raising doubts as to whether the 'open window' was open for a sufficient period of time to account for the increased susceptibility to infection (Brolinson & Elliot, 2007; Córdova, Sureda, Tur & Pons, 2010). Prolonged or repeated bouts of high intensity exercise can inhibit the production of immunoglobins by B – lymphocytes and reduce antigen-presenting cell function resulting in the ‘open window’ being exposed for a longer period (Harper Smith, Coakley, Ward, Macfarlane, Friedmann & Walsh, 2011). Periods of intensified training

lasting a week or more are common during a premier league season (Mohr, Bangsbo & Krustrup, 2003). The accumulative effects of minor changes in several immune factors may compromise resistance to common infections and illnesses, particularly during periods of repeated intense training and regular matches. In their position statement on immune function and exercise, Gleeson and Walsh (2012) stated that to date the only immune measure to show consistent relationships with URTI symptoms in exercising populations is changes in sIgA concentrations and secretion rates. Alongside salivary sampling, blood sampling using urea and CK has been used by sports medicine practitioners to monitor the athletes they work with (Fragala, Kraemer, Denegar, Maresh, Mastro & Volek, 2011; Heisterberg, Fahrenkrug & Andersen, 2013).

Saliva sampling

Recently several studies have advocated the use of saliva sampling as a diagnostic tool for measuring athletes hormonal, endocrine and immune responses to training and match play (Moreira, Arsati, Arsati, Da Silva & de Araújo, 2009; Moreira, Mortatti, Arruda, Freitas, de Arruda & Aoki, 2014; Silva, Rebelo, Marques, Pereira, Seabra, Ascensão & Magalhães, 2013). The use of saliva has gained popularity as it can be obtained quickly and frequently in a non-invasive manner (Papacosta, Gleeson & Nassis, 2013). Salivary IgA and cortisol testing has been used extensively to monitor the physiological and immune response to match play and training load in elite athletes (Papacosta & Nassis, 2011). One of the primary reasons salivary cortisol is used is due to it being proposed as a superior indicator than blood

samples as it represents the biologically active, free fraction of blood cortisol (Gozansky, Lynn, Laudenslager & Kohrt, 2005). Studies have also shown that cortisol responses to exercise are more pronounced in salvia than compared to blood, therefore offering a more accurate measure for the assessment of dynamic hypothalamic-pituitary-adrenal (HPA) axis activity (Crewther, Lowe, Ingram & Weatherby, 2010). As the predominant immunoglobin expressed in mucosal fluids, sIgA is widely considered the optimal indicator of mucosal immunity (Papacosta & Nassis, 2011). He, Tsai, Ko, Chang and Fang (2010) reported a decreased concentration and secretion rate of sIgA is associated with increased salivary cortisol levels, which may indicate that elevated cortisol levels may serve as a pre - cursor for suppression of mucosal immunity. Further research has also noted the relationship between sIgA and cortisol and concluded that monitoring these biomarkers can be a useful tool in the evaluation of individual recovery status, potential immune suppression and overreaching symptoms (Clements, 2013).

Blood sampling

The physical load that soccer players are subject to during a complete premier league season can cause critical changes in haematological factors (Heisterberg, Fahrenkrug, Krustrup, Storskov, Kjær & Andersen, 2013). Increases in CK and urea levels in response to strenuous exercise may be a consequence of both metabolic and mechanical stress (Brancaccio, Maffulli & Limongelli, 2007). The level of skeletal muscle enzymes has been reported as a reliable biomarker of the functional status of muscle tissue (Brancaccio, Maffulli & Limongelli, 2007). An increase in these enzymes has regularly been

seen in normal subjects and athletes after strenuous exercise and in particular, eccentric loading (Kenney, Landau, Gonzalez, Hundertmark, O'Brien & Campbell, 2012). In the event of exercise induced muscle damage, cell membranes are damaged and proteins leak out into the bloodstream with researchers analysing these using methods such as CK, myoglobin and lactate dehydrogenase testing (Heisterberg, Fahrenkrug, Krustrup, Storskov, Kjær & Andersen, 2013). Creatine Kinase has been the most extensively studied indicator of the physiological status of the muscle in elite athletes (Hunkin, Fahrner & Gastin, 2014; Lazarim et al. 2009). High levels of CK in healthy subjects may be correlated with training status, whereas if these levels persist at rest it may be an indication of non-functional overreaching syndrome or inadequate dietary intake (Baird, Graham, Baker & Bickerstaff, 2012). Urea is a marker of both enhanced nucleotide cycle turnover and the breakdown of amino acids (Viru & Viru 2001). It has repeatedly been shown that CK and urea are increased and remained elevated several days after a single soccer game in adult male players (Ascensão, Rebelo, Oliveira, Marques, Pereira & Magalhães, 2008; Ispirlidis et al. 2008; Fatouros et al. 2010).

Establishing data for players' recovery markers could enable coaches to make more informed decisions on training load and recovery to maximise player availability (Heisterberg, Fahrenkrug, Krustrup, Storskov, Kjær & Andersen, 2013). Having knowledge of when in the season increased stress to the physiological and endocrinal systems occurs may allow for –

- Improved planning of nutrition strategies to enhance recovery

- Better planning of training volume and intensity at these times
- Incorporation of more recovery sessions
- Use of other players to minimise fatigue
- Identification of when a potential 'break' would be best implemented (warm weather or winter break)

Despite recent research investigating the acute responses of biomarkers in elite soccer players, the variations over an entire season are poorly understood. It is possible that different times during the season result in specific physiological responses, therfore recording biomarker data could provide the 'recovery status' and 'readiness to train' information on players which is beneficial to both sports medicine and coaching staff (Owen, Wong, Dunlop, Groussard, Kebsi, Dellal, Morgans & Zouhal, 2014).

Creatine Kinase

Creatine Kinase (CK) is an enzyme that is routinely used as a biomarker for the metabolic changes in the muscle cells in response to match play and training loads (Ehlers, Ball & Liston, 2002). CK is active in the phosphocreatine (PCr) energy pathway catalysing the conversion of creatine and ATP to PCr and adenosine diphosphate (ADP) (Baird, Graham, Baker & Bickerstaff, 2012). Alongside being involved in energy production, CK can be raised from the damage of muscle tissue as a consequence of strenuous exercise (Mougios, 2007). Exercise damages the cell structure at the level of the sarcolemma and Z-disk, resulting in increased cell membrane permeability (Hody, Rogister, Leprince, Wang & Croisier, 2013). Mild to moderate exercise

has little influence on the cell membrane but as the intensity of exercise increases, CK leaks into the interstitial fluid and is removed by the lymphatic system (Ascensão, Rebelo, Oliveira, Marques, Pereira & Magalhães, 2008). Numerous factors determine the degree of enzyme activity post exercise. Weight bearing exercise that predominately includes a high number of eccentric muscle contractions has been reported to result in high CK activity (Baird, Graham, Baker & Bickerstaff, 2012). The need to establish baseline values and trends of CK levels over a season for individuals is highlighted by research suggesting that subjects can be classified as high or low responders (Totsuka, Nakaji, Suzuki, Sugawara & Sato, 2002). Brancaccio, Maffulli and Limongelli (2007) reported that there is a breakpoint of CK release in the muscle cell at 300-500 IU/L post exercise with the levels of enzyme release being correlated with individual muscular qualities. Mougios (2007) reported that highly trained athletes respond with lower CK levels compared to untrained subjects, but daily training without sufficient recovery can result in consistently high CK values (Heisterberg, Fahrenkrug, Krustrup, Storskov, Kjær & Andersen, 2013). Due to the high frequency of matches during a premier league season, which include a large number of eccentric contractions and decelerations, monitoring CK levels could provide a valuable insight into the physiological state of the players post game. Lazarim et al. (2009) conducted a study analysing the changes in CK levels in 128 professional soccer players at different times during the Brazilian national championship. The authors identified the upper limit reference value of the 90^{th} percentile (950IU/L) as the decision limit to detect muscle overload. A total of 6 players exceeded this value and were asked to decrease their sport activity levels, with their CK

being recorded a week later. All CK levels decreased as shown by table 1, with the subjects returning to their training schedule without any further reported problems.

Table 1. Creatine Kinase levels of the 6 players who displayed high levels. (Lazarim et al. (2009).

Athletes	CK Values (IU/L)	
	1	2
A	1316	729
B	1090	482
C	983	513
D	1245	507
E	1340	687
F	1800	Not Done
2 - Analysis performed after 1 week of reduced activity		

#The authors concluded that establishing an upper limit for CK activity and measuring players CK values against this may be beneficial for the early detection of muscular overload or fatigue. This protocol could be beneficial alongside determining players' individual mean values over the season. Ascensão, Rebelo, Oliveira, Marques, Pereira & Magalhães (2008) analysed the effect of a soccer match on plasma levels of oxidative and muscle damage markers. Sixteen professional soccer players' blood samples were taken at 30 minutes, 24, 48 and 72 hours post match. Figure 1 displays the behaviour of CK over these time points.

Figure 1. Creatine Kinase levels over the 5 time points post match. (Ascensão, Rebelo, Oliveira, Marques, Pereira & Magalhães, 2008).

It can be seen that there is a peak in CK levels at 48 hours post match. If a soccer team has one game per week then adequate recovery can be scheduled into the training week to allow for optimal muscle recovery, however if there are two games per week then the expectation to train at 48 hours will result in the players exercising with peak CK levels. The research conducted by Kingsley, Wadsworth, Kilduff, McEneny and Benton (2005) provided similar results in that a peak in CK activity was observed at 24 hours after exhaustive intermittent exercise in sixteen soccer players. Whilst most authors have verified increases in CK after a period of intense training, Silva, Santhiago, Papoti and Gobatto (2008) observed no changes in CK over a 12-week training period in 15 soccer players. The authors proposed that the difference in training intensities, alongside structured recovery sessions that were implemented into the training program contributed to the contrast in results

to other research. Meister, Faude, Ammann, Schnittker and Meyer (2013) conducted a comprehensive study tracking biomarkers over a competitive season in elite soccer players. The design included two, three week periods of high match exposure and low match exposure were compared with CK levels showing no significant alterations between the high and low exposures. Further investigations into the variations of CK in response to the load elite soccer players are exposed to, alongside more longitudinal studies would be beneficial. This would enhance the understanding of when in the season strategies could be implemented to maximise performance and recovery.

Urea

Urea is produced via protein breakdown and is formed in the liver when nitrogen, in the form of ammonia, combines with other substrates. Urea is released into the bloodstream, filtered by the kidneys and excreted in the urine. Monitoring urea levels can provide information on multiple physiological markers linked to recovery from exercise (Meyer & Meister, 2011). Research specifically conducted on soccer players or elite athletes is scarce. The aforementioned study by Meister, Faude, Ammann, Schnittker and Meyer (2013) also tracked urea levels over the season with no significant alterations between the high and low match exposures being observed. These results are in accordance with the study conducted by Silva, Santhiago, Papoti and Gobatto (2008) who reported that urea concentrations did not demonstrate any changes in response to a 12-week soccer-training program in fifteen players. However Andersson, Raastad, Nilsson, Paulsen, Garthe and Kadi (2008) observed a significant increase ($p < 0.05$) in urea after a

competitive match in elite female soccer players. Urea can also be used as a marker of increased nucleotide cycle turnover and the breakdown of amino acids (Viru & Viru, 2001). A further cause of excessive urea reading can be dehydration, which can easily occur during strenuous exercise or improper rehydration following a match (Mohr et al. 2010). With regard to these factors, high urea levels can be remedied through correction of diet and hydration strategies, both of which are integral to optimal performance in elite soccer players (Meyer & Meister, 2011).

Cortisol

The body's response to exercise stress is dependent on multiple systems interacting to maximise energy production alongside maintaining a state of homeostasis (Brownlee, Moore & Hackney, 2005). This adaptation to physical or psychological stress involves the activation of the adrenal glucocorticoids, hypothalmic corticotropin releasing hormone and anterior pituitary adrenocorticotropic hormone (Moreira, Arsati, Arsati, da Silva & de Araújo, 2009). These hormonal and neuroendocrine systems contribute to the both the short term homeostatic control and chronic cellular adaptation to exercise stress (Kraemer & Ratamess, 2005). Acute responses to exercise include an elevation in circulating hormone concentrations with cortisol secretion via the HPA axis increasing in proportion to exercise intensity (Moreira, Arsati, Arsati, da Silva & de Araújo, 2009). Cortisol is considered the primary hormone responsible for the rapid mobilisation of amino acids and fat to be utilised as energy sources (Hill, Zack, Battaglini, Viru, Viru & Hackney, 2008). In conjunction with this process, cortisol contributes to the catabolic processes

of reduced protein synthesis, increased protein degradation and inhibiting the inflammatory process and immunity (Thorpe and Sunderland, 2012). Researchers have identified the fact that the hormonal response to exercise was different depending on the type and intensity of the exercise (Wahl, Zinner, Achtzehn, Bloch & Mester, 2010). Cortisol plays a central role in the physiological response to a physical challenge or to a psychological stressor (Hellhammer, Wüst, & Kudielka, 2009); however, consistently elevated or suppressed values can lead to undesirable outcomes (Kraemer & Ratamess, 2005).

Response of cortisol to exercise

Numerous studies have monitored the acute response of cortisol in response to training and competition in elite athletes. Beaven, Gill and Cook (2008) investigated the cortisol responses to different resistance exercise protocols. The study reported a significant decrease in cortisol (44.3% ± 20.6%; $p < 0.01$) in response to a 3 × 5 at 40% of 1 repetition maximum protocol. This data is in contrast to the majority of the current research that suggests an acute increase in cortisol is observed in response to an increase in volume and/or intensity (Brownlee, Moore & Hackney, 2005). Crewther, Cronin, Keogh and Cook (2008) investigated the cortisol and testosterone responses to 3 different resistance-training schemes. The authors established that the hypertrophy workout program elicited a significant increase in cortisol concentrations (47% to 290%; $p \leq 0.05$) compared to the strength and power training programs even with the same workout duration being performed. The same researcher also examined the relationship between neuromuscular performance and

salivary hormones in elite rugby union players. A correlation was observed between cortisol concentrations and players performance with higher cortisol concentration causing a reduction in neuromuscular output. Recent research by West et al. (2014) reported the neuromuscular function and hormonal responses to a professional rugby match. Cortisol concentrations increased from baseline to 12 hours (baseline 0.40 ± 0.09 μg·dl−1 vs. 12 h 0.60 ± 0.20 $\mu g \cdot dl^{-1}$; $p = 0.004$) and 36 hours (0.60 ± 0.20 $\mu g \cdot dl^{-1}$ $p = 0.027$) but were similar at 60 hours post-match. Multiple factors can influence cortisol responses which can make the interpretation of the data difficult (Papcosta & Nassis, 2011), however all researchers concluded that neuromuscular output and hormonal disruption is present for up to 60 hours post-match. Monitoring cortisol responses over a longer time period could offer results more applicable to the elite team-sporting environment.

Schelling, Calleja, and Terrados (2009) monitored hormonal responses at 8 time points in elite basketball players throughout a season. Samples were taken at 24-36 hours post-game with cortisol not showing significant variations throughout the season. The research by He, Tsai, Ko, Chang and Fang (2010) opposed this finding by stating that cortisol concentrations increased throughout the season. Cortisol was highest at the first time point and following the first two games of the season. The first time point was taken after 3 days of the pre-season training period. This data supports the hypothesis that cortisol secretion is associated with exercise intensity, increased psychological stress from competition and reduced adaptation of the HPA axis to exercise stress (Doan, Newton, Kraemer, Kwon & Scheet, 2007; Hill, Zack, Battaglini, Viru, Viru & Hackney, 2008). Monitoring the

hormonal responses over a season introduces other factors that may influence the results beyond exercise intensity and volume, with subjects diet, recovery strategies and even seasonal variations affecting the concentrations of cortisol (Thorpe & Sunderland, 2012). Even so, applying the high level of ecological validity of these findings to a competitive soccer environment will allow for more specific conclusions to be drawn.

Cortisol and soccer

Ispirlidis et al (2008) recently investigated the effect of a single soccer game on indices of performance, muscle damage and inflammation. With reference to the cortisol response, there was a significant difference between pre and post-game readings. Cortisol concentration increased immediately post-game and returned to baseline levels at 24 hours, however, the participants refrained from any strenuous exercise for at least 7 days before and after the game. The authors suggested that 72 hours was required for anaerobic and muscle damage markers to return to normal levels and recommended this time frame as the optimal recovery for elite soccer players returning to full training post game. In contrast to this study, the study performed by Moreira, Arsati, Arsati, da Silva and de Araújo (2009) investigated the salivary cortisol responses to a competitive training match in male professional soccer players. Although they observed a trend of an increase in salivary cortisol from pre-match to post-match, the results were not statistically significant. The researchers suggested that multiple factors could influence the secretion of cortisol in elite soccer players. The importance of the match alongside the psychological component of competition was postulated as a reason for the

lack of an increase post game. The authors also commented on the individual variability of the responses with a recommendation for the need to establish more detailed data with regard to cortisol responses within team sports. Rimmele et al. (2007) observed that elite sportsmen displayed reduced reactivity to a standardised psychological laboratory stressor (trier social stress test) than untrained subjects. Cortisol responses were significantly lower ($p \leq 0.05$) for the elite sportsmen with the authors concluding that physical activity may provide a protective effect against stress related disorders. This study underlines the need to individualise the interpretation of the biomarkers recorded as multiple factors can influence hormonal responses to exercise.

Da Silva, Papoti, Santhiago, Pauli and Gobatto (2011) commented that an increment in training intensity plays an important role in cortisol responses. They took samples from 18 professional soccer players at three different time periods over a 12 week training cycle with cortisol displaying significantly higher values ($p \leq 0.05$) at 6 weeks (554.6±95.3 $nmol.L^{-1}$) and 12 weeks (612.2±115.8 $nmol.L^{-1}$) as compared to the beginning of the training (442.9±95.1 $nmol.L^{-1}$). The increase in cortisol from time period 1- time period 2 and time period 1- time period 3 was 23.1% and 43.2% respectively. This research is in accordance to that of Minetto, Lanfranco, Tibaudi, Baldi, Termine and Ghigo (2008) who proposed that the increase in cortisol levels may be explained by a hyper responsiveness of the HPA axis due an adaptation of the neuroendocrine system to chronic exercise demands. Using two different indexes, awakening cortisol response and midnight salivary cortisol, they examined the effect of a 7-day intensified training period on HPA axis

activity in recreational soccer players. They found a significant increase in post-training awakening cortisol response vs. pre-training awakening cortisol response ($p < 0.001$) after the 7-day training period. Midnight salivary cortisol also displayed a significant increase after training (before: 3.0 ± 0.7 vs. after: 5.9 ± 3.3 nmol.L^{-1}; $p = 0.017$). The authors concluded that further studies are required to clarify the physiological factors which underlie the exercise induced changes in cortisol responses and their value in predicting impaired adaptations to exercise. Moreira, de Moura, Coutts, Costa, Kempton and Aoki (2013) monitored the relationships of salivary cortisol and sIgA over a 4-week period during the competitive season in elite Futsal players. Samples were collected once per week with the researchers reporting that contrary to their hypothesis, there were no significant changes in rest salivary cortisol concentrations during the training period. The study took place during the competitive season but only one game was played during the 3rd week of the investigation. Considering the timing of the study, the players involved may well have adapted to the stress of training and this could explain why there was no change in cortisol levels. Furthermore with only one official match being played over the four week period the psychological stress of competition is absent. The authors suggested that although resting cortisol levels may provide an insight into the function of the HPA axis during intensive training, the cortisol response to a physical training load is extremely variable. Additionally the authors suggested that a longer period of training with a more frequent saliva sampling protocol should be investigated within elite soccer players to determine the pattern of cortisol response.

Kraemer et al. (2004) conducted a study that assessed the hormonal responses and physical performance measures at five different time points over an 11-week season in male collegiate soccer players. Results from the study displayed a significant decrease in sprint speed (- 4.3%) and vertical jump (-13.8%) at the 4^{th} time point. Concentrations of cortisol were elevated during the study compared to baseline and remained at the high end of normal range (138-635 $nmol \cdot^{-1}$) into the final testing date. The authors commented that players who enter the season with elevated cortisol concentrations could experience reductions in performance during the season. The initiation of a catabolic environment during the pre-season period due to an increased training load is something that requires close monitoring to ensure this is not carried into the competitive season. Apart from the research conducted by Kraemer et al. (2004), there is limited data on the seasonal variations in cortisol in elite soccer. The previously mentioned study by He, Tsai, Ko, Chang and Fang (2010) monitored the relationship of physiological biomarkers in elite basketball players over a season and reported that secretion rates and absolute concentrations of cortisol were significantly increased during the training and competition periods with an inverse correlation between sIgA and cortisol is present (r = -0.28; $p \leq 0.05$). As with all studies monitoring the hormonal responses of athletes over a significant time period, interpreting and applying the data has to be done with caution due to the difference in research designs (Moreira, Arsati, Arsati, da Silva and de Araújo, 2009). However, the relationship between cortisol and sIgA is one that researchers have increasiningly been using to monitor the physiological status of elite athletes.

Salivary immunoglobin A

Mucosal immunity constitutes the first line of defence against pathogen invasion and approximately 95% of all reported URTI's are initiated at the mucosal surfaces. Salivary immunoglobulin A (sIgA) is considered the best indicator of mucosal immunity, as it is the predominant antibody expressed in mucosal fluids (Papacosta & Nassis, 2011). It is thought that intense exercise will cause a suppression of sIgA levels potentially leading to an increase in the susceptibility of infection and specifically URTI (Gleeson, Pyne & Callister, 2003). However, studies have shown that moderate exercise can increase sIgA concentrations therefore decreasing the risk of URTI (Gleeson and Walsh, 2012). As previously stated the 'open window' that occurs post exercise is also relevant to sIgA with research showing a decrease in sIgA levels for up to 4 days post exercise (Neville, Gleeson & Folland, 2008; Nieman & Nehlsen-Cannarella, 1991). As part of the innate immune response, sIgA has been suggested as a marker to assist in the evaluation of excessive training loads, individual recovery status and assessment of potential immune suppression (Tiollier, Gomez-Merino, Burnat, Jouanin, Bourrilhon, Filaire & Chennaoui, 2005). Multiple studies have reported a decrease in sIgA concentrations in response to intense exercise in sports such as swimming (Gleeson, McDonald, Pyne, Clancy, Cripps, Francis and Fricker, 2000), basketball (He, Tsai, Ko, Chang and Fang, 2010) and American football (Fahlman and Engels, 2005). Recently there has been an increase in the number of soccer specific studies utilising sIgA testing to monitor match and training responses.

Salivary immunoglobin A and soccer

Moreira, Arsati, de Oliveira Lima-Arsati, de Freitas and de Araujo (2011) investigated the responses of sIgA in 10 professional top-level Brazilian futsal players after two highly competitive matches separated by seven days. Results from the study showed that a competitive training match produced a decrease in sIgA concentrations with the authors concluding that this can increase the vulnerability to infections meditated by the training and match stimulus. The same author more recently examined the changes in sIgA, cortisol, and URTI and their relationships with training loads during a 4-week period of intensive training during the season in elite Brazilian futsal players. Interestingly, no significant differences were observed for cortisol or sIgA during the study. The absence of a competitive match in the second study could have contributed as to why conflicting results were observed. Supporting this finding was the study performed by Moreira, Arsati, Cury, Franciscon, Oliveira and Araujo (2008) with a 70-minute non-competitive soccer match resulting in no changes in sIgA being observed. In contrast to these findings, an increase in sIgA concentrations during single and repeated bouts of soccer specific exercise was observed by Sari-Sarraf, Reilly, Doran and Atkinson (2007). Fredericks, Fitzgerald, Shaw and Holt (2012) investigated the relationship between sIgA and football training and match play involving elite-level footballers. Samples were taken prior to training; 20 minutes post session and then 18 hours after the session the following morning. The match play samples were collected from players at two time points: 20 minutes after the games and at 8:00 am the following morning (same for both home and away games). The results showed there was a significant fall in sIgA ($p <$ 0.001) between the pre and post 20-minute periods, which returned to pre-

training values following overnight rest. This was in contrast to the overnight rest following the two matches. The ten hours of rest following the second game was not sufficient for sIgA to return to the pre-match levels. The match play investigation, involving two successive competitive football matches, showed a significant fall in sIgA ($p < 0.0001$) following the second match, as compared to pre-match values for that match. This study emphasises the nature of a soccer season with the minimal recovery periods that are available. Two recent studies have further examined the sIgA responses in soccer players. Owen et al (2014) examined sIgA responses to different training intensity sessions (low intensity vs. high intensity sessions). The objective of the study was to try and identify key variables (e.g. GPS data, RPE, training duration) that could affect sIgA. Saliva samples of ten elite professional soccer players were collected before the investigation started to establish the baseline levels and before and after each training session. The principal finding of the investigation was that a significant reduction in sIgA values ($p < 0.05$) were observed in the post-exercise window following the completion of high intensity training when compared against low intensity training. Interestingly, the fourth period of training showed the largest decrease in sIgA concentrations for the high intensity training compared to the low intensity ($p < 0.05$) suggesting that repeated exposure to high intensity training loads could lead to increased fatigue and immune suppression.

Moreira, Mortatti, Arruda, Freitas, de Arruda and Aoki (2014) investigated sIgA response and URTI symptoms during a 21-week competitive season in young soccer players. This incorporated a pre-season training phase, a 7-week

competitive block with a 2-week de-training phase. The main finding of this study was that significant increase in sIgA concentrations ($p < 0.05$) being observed after the detraining phase, which mirrored the decrease in URTI symptom scores. The authors concluded that a short prophylactic recovery period induces improvements in the primary marker of mucosal immunity alongside reducing the risk of URTI. This novel finding emphasises the value of monitoring the physiological biomarkers of recovery in athletes to improve the protocols and interventions that are implemented by sports medicine, sports science and coaching staff.

Conclusion

The pursuit for marginal gains that can influence an athlete's health, performance or recovery status is one that it is high demand. Acquiring knowledge of the acute, transient and chronic responses of multiple physiological markers to training and match load could provide valuable information to aid the prescription of an athletes program (Meister, Faude, Ammann, Schnittker & Meyer, 2013). Using multiple methods to monitor athletes' physiological status via blood and saliva ensures that a comprehensive profile can be developed. The discrepancies in the research highlight the need for more practical, longer-term studies to be performed. Monitoring the variations over intense periods of the season (pre-season, Christmas, end of the season) is an attractive area of research as these time points are crucial with regard the overall season. Even so, using the data available specific, targeted interventions can be implemented on a team and individual basis. With specific note to elite athletes, any marginal gain could

lead to improved performance, with the use of saliva and blood sampling providing an attractive tool to aid in achieving this.

References

Ascensão, A., Rebelo, A., Oliveira, E., Marques, F., Pereira, L., & Magalhães, J. (2008). Biochemical impact of a soccer match—analysis of oxidative stress and muscle damage markers throughout recovery. *Clinical Biochemistry*, *41*(10), 841-851.

Baird, M. F., Graham, S. M., Baker, J. S., & Bickerstaff, G. F. (2012). Creatine-kinase-and exercise-related muscle damage implications for muscle performance and recovery. *Journal of Nutrition and Metabolism*, 960363-960363.

Beaven, C. M., Gill, N. D., & Cook, C. J. (2008). Salivary testosterone and cortisol responses in professional rugby players after four resistance exercise protocols. *The Journal of Strength and Conditioning Research*, *22*(2), 426-432.

Bloomfield, J., Polman, R., & O'Donoghue, P. (2007). Physical demands of different positions in FA Premier League soccer. *Journal of Sports Science and Medicine*, *6*(1), 63.

Bradley, P. S., Sheldon, W., Wooster, B., Olsen, P., Boanas, P., & Krustrup, P. (2009). High-intensity running in English FA Premier League soccer matches. *Journal of Sports Sciences*, *27*(2), 159-168.

Brancaccio, P., Maffulli, N., & Limongelli, F. M. (2007). Creatine kinase monitoring in sport medicine. *British Medical Bulletin*, *81*(1), 209-230.

Brolinson, P. G., & Elliott, D. (2007). Exercise and the immune system. *Clinics in Sports Medicine*, *26*(3), 311-319.

Brownlee, K. K., Moore, A. W., & Hackney, A. C. (2005). Relationship between circulating cortisol and testosterone: influence of physical exercise. *Journal of Sports Science and Medicine*, *4*(1), 76.

Clements, A. D. (2013). Salivary cortisol measurement in developmental research: Where do we go from here? *Developmental Psychobiology*, *55*(3), 205-220.

Córdova, A., Sureda, A., Tur, J. A., & Pons, A. (2010). Immune response to exercise in elite sportsmen during the competitive season. *Journal of Physiology and Biochemistry*, *66*(1), 1-6.

Crewther, B., Cronin, J., Keogh, J., & Cook, C. (2008). The salivary testosterone and cortisol response to three loading schemes. *The Journal of Strength and Conditioning Research*, *22*(1), 250-255.

Crewther, B. T., Lowe, T. E., Ingram, J., & Weatherby, R. P. (2010). Validating the salivary testosterone and cortisol concentration measures in response to short high-intensity exercise. *The Journal of Sports Medicine and Physical Fitness*, *50*(1), 85-92.

Da Silva, A. S. R., Papoti, M., Santhiago, V., Pauli, J. R., & Gobatto, C. A. (2011). Serum and plasma hormonal concentrations are sensitive to periods of intensity and volume of soccer training. *Science and Sports*, *26*(5), 278-285.

Dellal, A., Chamari, K., Wong, D. P., Ahmaidi, S., Keller, D., Barros, R., ... & Carling, C. (2011). Comparison of physical and technical performance in

European soccer match-play: FA Premier League and La Liga. *European Journal of Sport Science*, *11*(1), 51-59.

Doan, B. K., Newton, R. U., Kraemer, W. J., Kwon, Y. H., & Scheet, T. P. (2007). Salivary cortisol, testosterone, and T/C ratio responses during a 36-hole golf competition. *International Journal of Sports Medicine*, *28*(6), 470-479.

Ehlers, G. G., Ball, T. E., & Liston, L. (2002). Creatine kinase levels are elevated during 2-a-day practices in collegiate football players. *Journal of Athletic Training*, *37*(2), 151.

Fahlman, M. M., & Engels, H. J. (2005). Mucosal IgA and URTI in American college football players: a year longitudinal study. *Medicine and Science in Sports and Exercise*, *37*(3), 374-380.

Fatouros, I. G., Chatzinikolaou, A., Douroudos, I. I., Nikolaidis, M. G., Kyparos, A., Margonis, K., ... & Jamurtas, A. Z. (2010). Time-course of changes in oxidative stress and antioxidant status responses following a soccer game. *The Journal of Strength and Conditioning Research*, *24*(12), 3278-3286.

Fragala, M. S., Kraemer, W. J., Denegar, C. R., Maresh, C. M., Mastro, A. M., & Volek, J. S. (2011). Neuroendocrine-immune interactions and responses to exercise. *Sports Medicine*, *41*(8), 621-639.

Fredericks, S., Fitzgerald, L., Shaw, G., & Holt, D. W. (2012). Changes in Salivary Immunoglobulin A (IgA) following Match-play and Training among

English Premiership Footballers. *Medicine Journal of Malaysia*, *67*(2), 155.

Gleeson, M., McDonald, W. A., Pyne, D. B., Clancy, R. L., Cripps, A. W., Francis, J. L., & Fricker, P. A. (2000). Immune status and respiratory illness for elite swimmers during a 12-week training cycle. *International Journal of Sports Medicine*, *21*(04), 302-307.

Gleeson, M., Pyne, D. B., & Callister, R. (2003). Exercise effects on mucosal immunity and risk of upper respiratory illness: review article. *International Sports Medicine Journal*, *4*(3), 1-14.

Gleeson, M., & Walsh, N. P. (2012). The BASES expert statement on exercise, immunity, and infection. *Journal of Sports Sciences*, *30*(3), 321-324.

Gozansky, W. S., Lynn, J. S., Laudenslager, M. L., & Kohrt, W. M. (2005). Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic–pituitary–adrenal axis activity. *Clinical Endocrinology*, *63*(3), 336-341.

Harper Smith, A. D., Coakley, S. L., Ward, M. D., Macfarlane, A. W., Friedmann, P. S., & Walsh, N. P. (2011). Exercise-induced stress inhibits both the induction and elicitation phases of in vivo T-cell-mediated immune responses in humans. *Brain, Behavior and Immunity*, *25*(6), 1136-1142.

He, C. S., Tsai, M. L., Ko, M. H., Chang, C. K., & Fang, S. H. (2010). Relationships among salivary immunoglobulin A, lactoferrin and cortisol in basketball players during a basketball season. *European Journal of Applied Physiology*, *110*(5), 989-995.

Heisterberg, M. F., Fahrenkrug, J., Krustrup, P., Storskov, A., Kjær, M., & Andersen, J. L. (2013). Extensive monitoring through multiple blood samples in professional soccer players. *The Journal of Strength and Conditioning Research*, *27*(5), 1260-1271.

Hellhammer, D. H., Wüst, S., & Kudielka, B. M. (2009). Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology*, *34*(2), 163-171.

Hill, E. E., Zack, E., Battaglini, C., Viru, M., Viru, A., & Hackney, A. C. (2008). Exercise and circulating cortisol levels: the intensity threshold effect. *Journal of Endocrinological Investigation*, *31*(7), 587-591.

Hody, S., Rogister, B., Leprince, P., Wang, F., & Croisier, J. L. (2013). Muscle fatigue experienced during maximal eccentric exercise is predictive of the plasma creatine kinase (CK) response. *Scandinavian Journal of Medicine and Science in Sports*, *23*(4), 501-507.

Hunkin, S. L., Fahrner, B., & Gastin, P. B. (2014). Creatine kinase and its relationship with match performance in elite Australian Rules football. *Journal of Science and Medicine in Sport*, *17*(3), 332-336.

Ispirlidis, I., Fatouros, I. G., Jamurtas, A. Z., Nikolaidis, M. G., Michailidis, I., Douroudos, I., ... & Taxildaris, K. (2008). Time-course of changes in

inflammatory and performance responses following a soccer game. *Clinical Journal of Sport Medicine*, *18*(5), 423-431.

Kenney, K., Landau, M. E., Gonzalez, R. S., Hundertmark, J., O'Brien, K., & Campbell, W. W. (2012). Serum creatine kinase after exercise: drawing the line between physiological response and exertional rhabdomyolysis. *Muscle and Nerve*, *45*(3), 356-362.

Kingsley, M. I., Wadsworth, D., Kilduff, L. P., McEneny, J., & Benton, D. (2005). Effects of phosphatidylserine on oxidative stress following intermittent running.*Medicine And Science In Sports And Exercise*, *37*(8), 1300-1306.

Kraemer, W. J., & Ratamess, N. A. (2005). Hormonal responses and adaptations to resistance exercise and training. *Sports Medicine*, *35*(4), 339-361.

Kraemer, W. J., French, D. N., Paxton, N. J., Häkkinen, K., Volek, J. S., Sebastianelli, W. J., ... & Knuttgen, H. G. (2004). Changes in exercise performance and hormonal concentrations over a big ten soccer season in starters and nonstarters. *The Journal of Strength and Conditioning Research*, *18*(1), 121-128.

Lazarim, F. L., Antunes-Neto, J. M., da Silva, F. O., Nunes, L. A., Bassini-Cameron, A., Cameron, L. C., ... & de Macedo, D. V. (2009). The upper values of plasma creatine kinase of professional soccer players during

the Brazilian National Championship. *Journal of Science and Medicine in Sport*, *12*(1), 85-90.

Malm, C, Ekblom, O, and Ekblom, B. (2004). Immune system alteration in response to two consecutive soccer games. *Acta Physiologica Scandinavica*, *180*(2), 143-155.

Meister, S., Faude, O., Ammann, T., Schnittker, R., & Meyer, T. (2013). Indicators for high physical strain and overload in elite football players. *Scandinavian Journal of Medicine and Science in Sports*, *23*(2), 156-163.

Meyer, T., & Meister, S. (2011). Routine blood parameters in elite soccer players. *International Journal of Sports Medicine*, *32*(11), 875-881.

Minetto, M. A., Lanfranco, F., Tibaudi, A., Baldi, M., Termine, A., & Ghigo, E. (2008). Changes in awakening cortisol response and midnight salivary cortisol are sensitive markers of strenuous training-induced fatigue. *Journal of Endocrinological Investigation*, *31*(1), 16-24.

Mohr, M., Krustrup, P., & Bangsbo, J. (2003). Match performance of high-standard soccer players with special reference to development of fatigue. *Journal of Sports Sciences*, *21*(7), 519-528.

Mohr, M., Mujika, I., Santisteban, J., Randers, M. B., Bischoff, R., Solano, R., ... & Krustrup, P. (2010). Examination of fatigue development in elite soccer in a hot environment: a multi-experimental

approach. *Scandinavian Journal Of Medicine and Science in Sports*, *20*(s3), 125-132.

Moreira, A., Arsati, F., Arsati, Y. B. D. O. L., da Silva, D. A., & de Araújo, V. C. (2009). Salivary cortisol in top-level professional soccer players. *European Journal of Applied Physiology*, *106*(1), 25-30.

Moreira, A., Arsati, F., de Oliveira Lima-Arsati, Y. B., de Freitas, C. G., & de Araujo, V. C. (2011). Salivary immunoglobulin A responses in professional top-level futsal players. *The Journal of Strength and Conditioning Research*, *25*(7), 1932-1936.

Moreira, A., de Moura, N. R., Coutts, A., Costa, E. C., Kempton, T., & Aoki, M. S. (2013). Monitoring internal training load and mucosal immune responses in futsal athletes. *The Journal of Strength and Conditioning Research*, *27*(5), 1253-1259.

Moreira, A., Mortatti, A. L., Arruda, A. F., Freitas, C. G., de Arruda, M., & Aoki, M. S. (2014). Salivary IgA Response and Upper Respiratory Tract Infection Symptoms During a 21-Week Competitive Season in Young Soccer Players. *The Journal of Strength and Conditioning Research*, *28*(2), 467-473.

Mortatti, A. L., Moreira, A., Aoki, M. S., Crewther, B. T., Castagna, C., de Arruda, A. F., & Jose Filho, M. (2012). Effect of competition on salivary cortisol, immunoglobulin A, and upper respiratory tract infections in elite young soccer players. *The Journal of Strength and Conditioning Research*, *26*(5), 1396-1401.

Mougios, V. (2007). Reference intervals for serum creatine kinase in athletes. *British Journal of Sports Medicine*, *41*(10), 674-678.

Neville, V., Gleeson, M., & Folland, J. P. (2008). Salivary IgA as a risk factor for upper respiratory infections in elite professional athletes. *Medicine And Science In Sports And Exercise*, *40*(7), 1228-1236.

Nieman, D. C., & Nehlsen-Cannarella, S. L. (1991). The effects of acute and chronic exercise on immunoglobulins. *Sports Medicine*, *11*(3), 183-201.

Nieman, D. C., Nehlsen-Cannarella, S. L., Markoff, P. A., Balk-Lamberton, A. J., Yang, H., Chritton, D. B. W., ... & Arabatzis, K. (1990). The effects of moderate exercise training on natural killer cells and acute upper respiratory tract infections. *International Journal of Sports Medicine*, *11*(06), 467-473.

Owen, L., Wong, D. P., Dunlop, G., Groussard, C., Kebsi, W., Dellal, A., ... & Cachan, F. (2014). High Intensity Training and Salivary Immunoglobulin-A Responses in Professional Top-Level Soccer Players: Effect of Training Intensity. *Journal of Strength and Conditioning Research,* [Epub ahead of print].

Papacosta, E., & Nassis, G. P. (2011). Saliva as a tool for monitoring steroid, peptide and immune markers in sport and exercise science. *Journal of Science and Medicine in Sport*, *14*(5), 424-434.

Papacosta, E., Gleeson, M., & Nassis, G. P. (2013). Salivary Hormones, IgA, and Performance During Intense Training and Tapering in Judo Athletes. *The Journal of Strength and Conditioning Research*, *27*(9), 2569-2580.

Rimmele, U., Zellweger, B. C., Marti, B., Seiler, R., Mohiyeddini, C., Ehlert, U., & Heinrichs, M. (2007). Trained men show lower cortisol, heart rate and psychological responses to psychosocial stress compared with untrained men. *Psychoneuroendocrinology*, *32*(6), 627-635.

Sari-Sarraf, V., Reilly, T., Doran, D. A., & Atkinson, G. (2007). The effects of single and repeated bouts of soccer-specific exercise on salivary IgA. *Archives of Oral Biology*, *52*(6), 526-532.

Schelling, X., Calleja, J., & Terrados, N. (2009). Hormonal analysis in elite basketball during a season. *Revista de Psicología del Deporte*, *18*(3), 363-367.

Silva, A. S. R., Santhiago, V., Papoti, M., & Gobatto, C. A. (2008). Psychological, biochemical and physiological responses of Brazilian soccer players during a training program. *Science and Sports*, *23*(2), 66-72.

Silva, J. R., Rebelo, A., Marques, F., Pereira, L., Seabra, A., Ascensão, A., & Magalhães, J. (2013). Biochemical impact of soccer: an analysis of hormonal, muscle damage, and redox markers during the season. *Applied Physiology, Nutrition and Metabolism*, *39*(4), 432-438.

Thorpe, R., & Sunderland, C. (2012). Muscle damage, endocrine, and immune marker response to a soccer match. *The Journal of Strength and Conditioning Research*, *26*(10), 2783-2790.

Tiollier, E., Gomez-Merino, D., Burnat, P., Jouanin, J. C., Bourrilhon, C., Filaire, E., ... & Chennaoui, M. (2005). Intense training: mucosal immunity and incidence of respiratory infections. *European Journal of Applied Physiology*, *93*(4), 421-428.

Totsuka, M., Nakaji, S., Suzuki, K., Sugawara, K., & Sato, K. (2002). Break point of serum creatine kinase release after endurance exercise. *Journal of Applied Physiology*, *93*(4), 1280-1286.

Viru, A. A., & Viru, M. (2001). *Biochemical Monitoring Of Sport Training*. Human Kinetics.

Wahl, P., Zinner, C., Achtzehn, S., Bloch, W., & Mester, J. (2010). Effect of high-and low-intensity exercise and metabolic acidosis on levels of GH, IGF-I, IGFBP-3 and cortisol. *Growth Hormone and IGF Research*, *20*(5), 380-385.

Walsh, N., Laing, S., Oliver, S., Montague, J., Walters, R. & Bilzon, J. (2004). Saliva parameters as potential indices of hydration status during acute dehydration. *Medicine and Science in Sports and Exercise*, *36*(9), 1535-1542

West, D. J., Finn, C. V., Cunningham, D. J., Shearer, D. A., Jones, M. R., Harrington, B. J., ... & Kilduff, L. P. (2014). Neuromuscular Function, Hormonal, and Mood Responses to a Professional Rugby Union Match. *The Journal of Strength and Conditioning Research*, *28*(1), 194-200.

Department of Clinical Sciences and Nutrition

MSc Exercise and Nutrition Science

Examining the biochemical and physiological responses in elite professional soccer players throughout a competitive season

Assessment Number: J12364

Word count: 4298

Key words: saliva analysis, blood analysis, soccer, match response.

The appropriate journal for the current article would be the Journal of Sport Sciences. It publishes research on various aspects of sport and exercise sciences, including human responses to exercise, performance prediction or modification, stress reduction, sports medicine and overall health in athletes. Manuscripts considered for publication include those dealing with original investigations of exercise, validation of innovations in sport or comprehensive reviews of topics relevant to the scientific study of sport. The current study encompasses the key stipulations required for publication in the journal.

Table of contents

Abstract

Background

During a premier league season there are considerable physical demands placed upon the players with huge emphasis on optimising recovery from both training and games. Saliva and blood sampling has been used as a diagnostic tool for the assessment of physiological biomarkers in response to training and match play. The purpose of the study is to examine the variations and relationships of specific salivary and blood biomarkers over a premier league season.

Method

The dependent variables salivary immunoglobin (sIgA), cortisol, blood urea and creatine kinase (CK) were measured to assess the physiological responses to competitive matches. Over the course of the season data samples were taken 16-18 hours after a competitive match at 9 seperate time points.

Results

Compared with time point 1 (T1), CK concentrations did not display any significant variation over the course of the season. Significant differences were observed in urea, sIgA and cortisol over the course of the season at specific time points. When compared with T1, there were significant increases in urea at T8 ($p < 0.05$) and T9 ($p < 0.05$). Similarly, significant increases in cortisol levels were observed at T8 ($p < 0.05$) and T9 ($p < 0.05$) compared to T1. Salivary IgA concentrations showed a significant increase at T8 ($p < 0.05$) when compared to T1. The results from the Pearson's correlation resulted in a

significant high positive correlation being observed between urea and cortisol (r = 0.76; p < 0.05).

Conclusion

The results of this study suggest that at the end of the season, significant physiological variations occur in response to match play. Using biomarkers can enable more purposeful and proactive interventions to maximise recovery and performance.

Introduction

Modern day soccer is classed as a high-intensity intermittent activity (Dellal et al. 2011). An outfield player can cover distances ranging from 10-13km (Bloomfield, Polman & O'Donoghue, 2007) whilst performing 1500 - 3000m of high intensity activity during a match (movement >18km/h) (Bradley, Sheldon, Wooster, Olsen, Boanas & Krustrup, 2009). The high level of physical activity coupled with the limited opportunity to schedule sufficient recovery may influence body homeostasis (Heisternerg, Fahrenkrug, Krustrup, Storskov, Kjær & Andersen, 2013). During a premier league season there are considerable physical demands as a result of training alongside the ever-increasing fixture congestion (Bloomfield, Polman & O'Donoghue, 2007). The measurement of physiological biomarkers in whole saliva and blood can offer a valuable tool for assessing the hormonal, immunological and endocrinological status associated with exercise and training (Ascensão, Rebelo, Oliveira, Marques, Pereira & Magalhães, 2008; Papacosta & Nassis, 2011). Failure to recover adequately from competitive games and training has been associated with the occurrence of immune suppression, a reduction in anaerobic performance and reduced neuromuscular output in soccer players (Da Silva, Papoti, Santhiago, Pauli and Gobatto, 2011; Malm, Ekblom & Ekblom, 2004; Sari-Sarraf, Reilly, Doran & Atkinson, 2008). Acute bouts of strenuous exercise can compromise various aspects of immune function by revealing an ‘open window’ of increased susceptibility to infection (Gleeson, Pyne & Callister, 2003). It is feasible that the accumulative effects of minor changes in several immune factors may compromise resistance to common infections and illnesses, particularly during periods of repeated intense

training and regular matches. In their position statement on immune function and exercise, Gleeson and Walsh (2012) stated that to date the only immune measure to show consistent relationships with URTI symptoms in exercising populations is changes in salivary immunoglobin (sIgA) concentrations and secretion rates. Alongside using sIgA, salivary cortisol, blood urea nitrogen (BUN) and CK sampling have been widely used by sports medicine practitioners to monitor the athletes they work with (Fragala, Kraemer, Denegar, Maresh, Mastro & Volek, 2011; Heisterberg, Fahrenkrug, Krustrup, Stroskov, Kjær & Andersen, 2013).

Saliva sampling

Recently several studies have advocated the use of saliva sampling as a diagnostic tool for measuring athletes hormonal, endocrine and immune responses to training and match play (Moreira, Mortatti, Arruda, Freitas, de Arruda & Aoki, 2014; Silva, Rebelo, Marques, Pereira, Seabra, Ascensão & Magalhães, 2013). The use of saliva has gained popularity as it can be obtained quickly and frequently in a non-invasive manner (Papacosta & Nassis, 2011). One of the primary reasons saliva is used is that by measuring the free concentrations it represents the biologically active portion of the hormone (Gozansky, Lynn, Laudenslager & Kohrt, 2005). As the predominant immunoglobin expressed in mucosal fluids, sIgA is widely considered the optimal indicator of mucosal immunity (Papacosta & Nassis, 2011). Current research studies have investigated the sIgA responses in soccer players during single match play and training bouts (Moreira, Arsati, de Oliveira Lima-Arsati,

de Freitas & de Araujo, 2011) and over a competitive season (Moreira, Mortatti, Arruda, Freitas, de Arruda & Aoki, 2014).

Cortisol plays a central role in the physiological response to a physical challenge or to a psychological stressor (Hellhammer, Wüst, & Kudielka, 2009); however, consistently elevated or suppressed values can lead to undesirable outcomes such as fatigue and non functional overreaching syndrome (Kraemer & Ratamess, 2005). It has been reported that salivary cortisol, as a representative of circulating free cortisol, can be used as an index of training stress (Papacosta, Gleeson & Nassis, 2013). Furthermore salivary cortisol has been proposed as a superior indicator than blood samples as it represents the biologically active, free fraction of blood cortisol (Gozansky, Lynn, Laudenslager & Kohrt, 2005). Both cortisol and sIgA responses to exercise are highly individual with researchers commenting that further studies examining the variation of these biomarkers over a longer time frame would be beneficial (Ispirlidis et al. 2008; Papcosta & Nassis, 2011).

Blood sampling

The physical load that soccer players are subject to during a complete premier league season can cause critical changes in hematological factors (Heisternerg, Fahrenkrug, Krustrup, Storskov, Kjær & Andersen, 2013). Increases in CK and urea levels in response to strenuous exercise may be a consequence of both metabolic and mechanical stress (Brancaccio, Maffulli & Limongelli, 2007). The level of skeletal muscle enzymes has been reported as a reliable biomarker of the functional status of muscle tissue (Brancaccio, Maffulli & Limongelli, 2007). In the event of exercise induced muscle damage,

and in particular, eccentric loading, cell membranes are damaged and proteins leak out into the bloodstream with researchers analysing these using methods such as CK testing (Heisternerg, Fahrenkrug, Krustrup, Storskov, Kjær & Andersen, 2013). Creatine Kinase (CK) has been the most extensively used indicator of the physiological status of the muscle in elite athletes (Hunkin, Fahrner & Gastin, 2014; Lazarim et al. 2009). High levels of CK in healthy subjects may be correlated with training status, but if these levels persist at rest it may be an indication of non-functional overreaching syndrome (Baird, Graham, Baker & Bickerstaff, 2012). Urea is a marker of both enhanced nucleotide cycle turnover and the breakdown of amino acids (Viru & Viru 2001). It has repeatedly been shown that CK and urea are increased and remained elevated several days after a single soccer game in adult male players (Ascensão, Rebelo, Oliveira, Marques, Pereira & Magalhães, 2008; Fatouros et al. 2010).

Establishing data for players' recovery markers could enable coaches to make more informed decisions on training load and recovery to maximise player availability (Heisternerg, Fahrenkrug & Andersen, 2014). Having knowledge of when in the season increased stress occurs may allow for –

- Improved planning of nutrition strategies to enhance recovery
- Better planning of training volume and intensity at these times
- Incorporation of more recovery sessions
- Use of other players to minimise fatigue

- Identification of when a potential ‘break’ would be best implemented (warm weather or winter break)

Despite recent research investigating the responses to various biomarkers in elite soccer players, the variations over an entire season are poorly understood. Recording this data could provide the ‘recovery status’ and ‘readiness to train’ information on players which is beneficial to both sports medicine and coaching staff (Owen, Wong, Dunlop, Groussard, Kebsi, Dellal, Morgans & Zouhal, 2014). Therefore, the primary purpose of this study was to examine the variation of these biomarkers over an entire season with a view to identifying key relationships.

It was hypothesised that accumulated demands of training and competition would lead to a temporal increase in cortisol levels with a concurrent reduction in sIgA concentrations. It was also hypothesised that CK and Urea would increase over the course of the season due to increased fatigue and regularity of the matches.

Method

Experimental Approach to the Problem

Providing information on the variance of muscle damage, immunity, and endocrine responses to competitive matches over the course of the season will help with the development of appropriate player recovery strategies, nutritional intervention and training load management. The dependent

variables sIgA, cortisol, blood urea, and CK were measured to assess the physiological responses to competitive matches. Over the course of the season a total of 820 salivary and blood samples were collected from 36 elite male soccer players between the 17th August 2013 and 30th April 2014. A total of 39 matches were played over the course of the sampling time frame. The data was restricted to samples that had been taken 16-18 hours after a competitive match. Nine time points were selected at the end of each representing a full competitive season. The time points selected will include the start of the season, the Christmas period, which included 4 matches in 9 days and the end of the season where the importance of the matches is amplified. Retrospective ethical approval was obtained from University of Chester's faculty of Life Sciences Ethics Committee.

Subjects

The data was further restricted to 10 players (4 defenders, 3 midfielders, 3 strikers (mean ± SD) stature 1.76 ± 7.88 m, mass 71.6 ± 8.52 Kg, age 27.1 ± 3.68 years) who had been tested at all time points. Figure 1 displays an overview of when in the season the samples were taken.

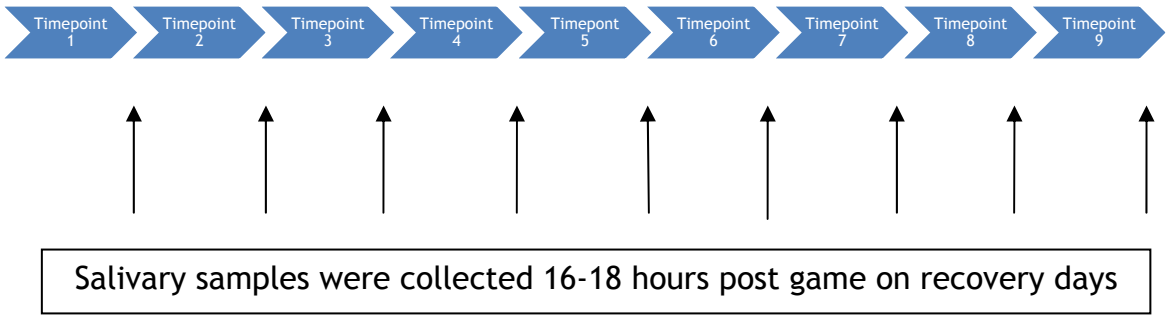

Figure 1. Graphic displaying sampling time frame

The players were required to undertake the testing procedure as part of their contract with the soccer club. All players were fully familiarised with the experimental procedures within the present study due to the testing protocols being implemented within the club as part of its medical and sports science structure. All testing data was collected 16-18 hours post game in the medical suite at the training ground. Samples were collected from 9 - 10.30am after breakfast was eaten but before any physical exercise was undertaken. Keeping the testing times at a consistent time of day is to minimise the effects of circadian variation on sIgA and cortisol (Hucklebridge, Clow & Evans, 1998).

Sampling Procedures

Saliva sampling was performed using an IPRO oral fluid collector (Dunbar, Jehanli, A & Browne, 2014). To regulate saliva secretion players were required to consume 500ml of water prior to sampling to ensure adequate hydration due to dehydration being linked with reduced resting saliva flow rates (Walsh, Laing, Oliver, Montague, Walters & Bilzon, 2004). The sampling procedure was followed in accordance to the manufacturers guidelines with players required to place a synthetic polymer-based swab attached to a volume adequacy indicator stem in their mouth. The IPRO OFC buffer contains extraction agents to draw the target analytes from the swab into the buffer. The sample was then analysed using a real time lateral flow device (LFD) (IPRO Interactive, Wallingford, UK). Separate cartridges were used to analyse sIgA and cortisol. The buffer solution was stored for 4 hours and then run for

analysis. This is in accordance to the manufacturers recommendations to reduce variability in the readings.

The blood test was undertaken in accordance with the standard operating procedure and training supplied by the manufacturer (Reflotron® Una Health, Burgess Hill, UK). The pad of the index finger was swabbed using an alcohol wipe before the sample was collected. A lancet device was placed against the side of index finger of the player and pressed to activate the lancet. The first blood was wiped away using a gauze swab to remove any skin cells or other substance that may influence the reading. The players' finger was then gently squeezed to encourage blood flow and a 32µl capillary tube was used to collect the blood. Once the full amount of blood was collected the capillary tube was placed into a pipette and the blood then placed onto the surface of a CK test strip (Reflotron® CK, Burgess Hill, UK). The CK test strip was placed immediately into the Reflotron plus analyser reader and the sample analysed. Once the reading had been recorded, the same pipette was used to place blood onto a separate urea test strip (Reflotron® Urea, Burgess Hill, UK), which was analysed suing the same machine.

Statistical analysis

The data were analysed using SPSS (version 21; SPPS, Inc, Chicago, IL, USA). The time points (T1, T2, T3 etc.) over the course of the season were used as the independent variable whilst the biomarkers CK, urea, sIgA and cortisol were the dependent variables. Data were expressed as mean values ±

standard deviation. Using the Shapiro-Wilk test data was assessed for normal distribution. All data presented with normally distribution, therefore multiple One-way repeated measure ANOVA with post hoc paired sample *t*-tests were used to analyse where the significant variations occurred. To assess the relationships between the dependent variables, a Pearson's correlation coefficient analysis was conducted. The level of significance was set at $p < 0.05$.

Results

Compared with T1, CK concentrations did not display any significant variation over the course of the season (figure 2). Significant differences were observed in urea, sIgA and cortisol over the course of the season at specific time points. As shown in figure 3, when compared with T1, there were significant increases in urea at T8 ($p < 0.05$) and T9 ($p < 0.05$). Similarly, significant increases in cortisol levels were observed at T8 ($p < 0.05$) and T9 ($p < 0.05$) compared to T1 (figure 4). As shown in figure 5, sIgA concentrations showed a significant increase at T8 ($p < 0.05$) when compared to T1. All data is presented in table 1.

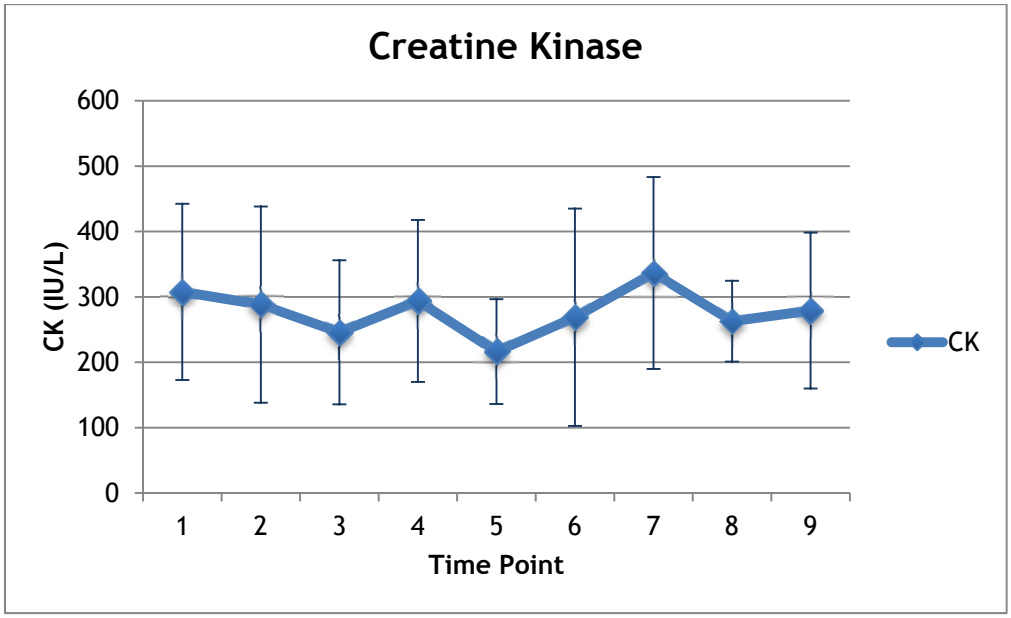

Figure 2. Changes in urea over the course of the season

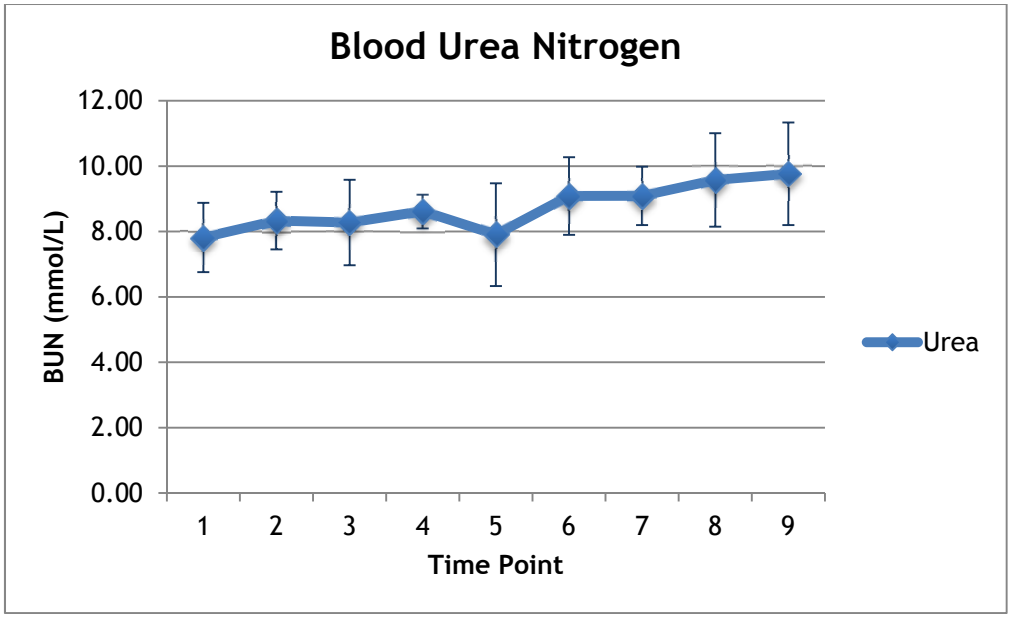

Figure 3. Changes in urea over the course of the season

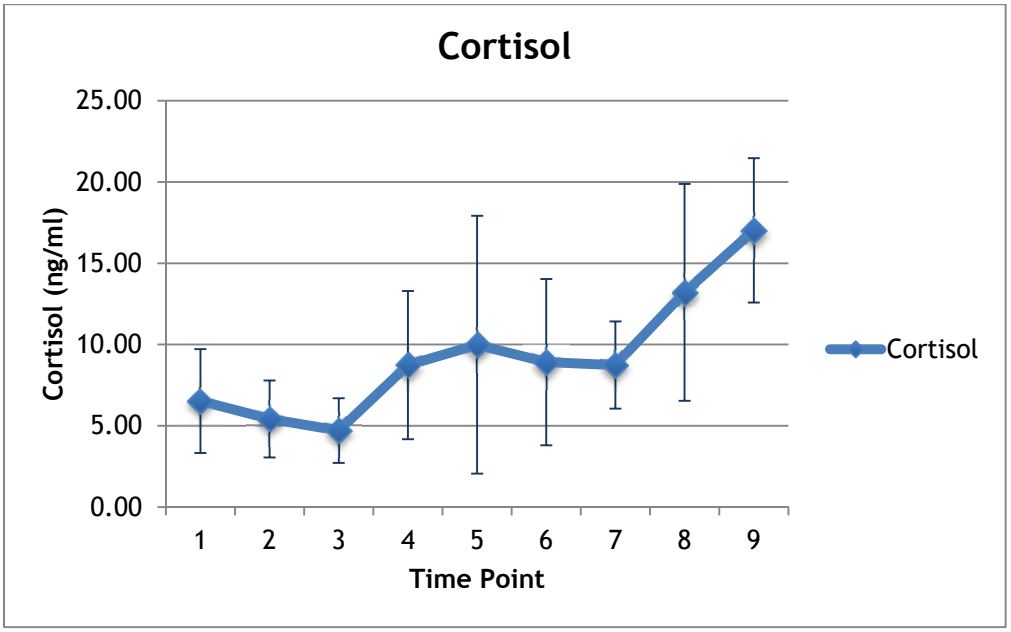

Figure 4. Changes in cortisol over the course of the season

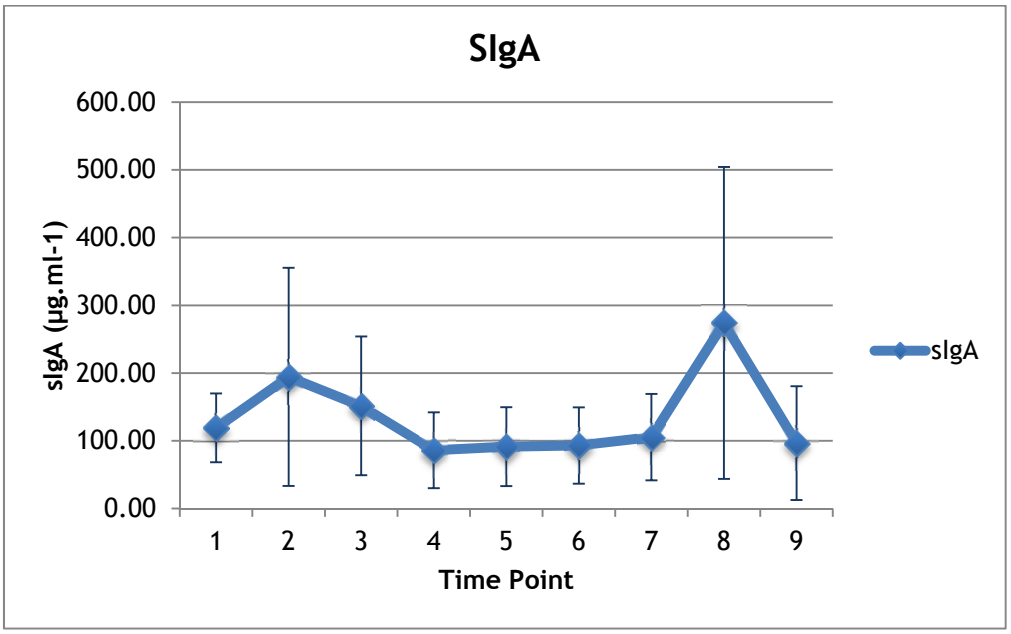

Figure 5. Changes in sIgA over the course of the season

Table 1. Changes in biomarkers over the course of the season

TimePoint	CK (IU/L)	Urea (mmol/L)	sIgA (µg.mL-1)	Cortisol (ng/ml)
1	308 ± 135	7.82 ± 1.1	119 ± 51	6.53 ± 3.2
2	288 ± 150	8.34 ± 0.9	194 ± 161	5.42 ± 2.4
3	246 ± 110	8.28 ± 1.3	152 ± 102	4.71 ± 2
4	294 ± 124	8.62 ± 0.5	86 ± 56	8.74 ± 4.6
5	216 ± 80	7.9 ± 1.6	91 ± 58	9.99 ± 7.9
6	269 ± 166	9.09 ± 1.2	93 ± 56	8.92 ± 5.1
7	337 ± 147	9.09 ± 1.2	105 ± 64	8.74 ± 2.7
8	263 ± 62	9.58 ± 1.4 *	274 ± 230 *	13.21 ± 6.7 *
9	279 ± 119	9.77 ± 1.6 *	97 ± 84	17.03 ± 4.4 *

Values are Mean ± SD

* $p < 0.05$, significant difference from T1

The results from the Pearson's correlation relationships between CK, urea, sIgA and cortisol of the soccer players during the season are displayed in Table 3. A high positive correlation was observed between urea and cortisol (r = 0.76; $p < 0.05$). No other significant correlations were observed between variables.

Table 2. Results from the Pearsons' correlation

		CK (IU/L)	Urea (mmol/L)	sIgA ($\mu g.mL^{-1}$)	Cortisol (ng/ml)
CK (IU/L)	Pearson Correlation	1	0.198	-0.098	-0.113
	Sig. (2-tailed)		0.609	0.801	0.772
Urea (mmol/L)	Pearson Correlation	0.198	1	.763*	0.222
	Sig. (2-tailed)	0.609		0.017	0.567
Cortisol (ng/ml)	Pearson Correlation	-0.098	.763*	1	0.009
	Sig. (2-tailed)	0.801	0.017		0.983
sIgA ($\mu g.mL^{-1}$)	Pearson Correlation	-0.113	0.222	0.009	1
	Sig. (2-tailed)	0.772	0.567	0.983	

* Correlation is significant at the 0.05 level (2-tailed).

Discussion

Due to the huge worldwide interest in Premier league soccer, it has become essential that the players receive optimal training, recovery, match preparation, diet and medical care to ensure peak physical performance is achieved (Filaire, Bernain, Sagnol & Lac, 2001). In this study, physiological and biochemical biomarkers were recorded over the course of a season to examine the variations in responses to 9 matches. The main finding of this study was that at T8, three of the biomarkers displayed significant changes compared to T1 ($p < 0.05$). Secondly, cortisol levels at T9 displayed the highest significant change ($p = 0.0001$) compared to T1. Furthermore, a

significant high positive correlation observed between urea and cortisol over the course of the season (r = 0.76; p < 0.05).

Salivary IgA

The principal finding with regard to sIgA was a significant increase in concentrations at T8 being observed compared with T1 (p = 0.04). No other statistically significant variations were observed over the course of the season. The findings of this study are in agreement with research showing an increase in the volume and intensity of exercise can cause suppression in sIgA levels (Gleeson, Pyne & Callister, 2003; Tiollier, Gomez-Merino, Burnat, Jouanin, Bourrilhon, Filaire & Chennaoui, 2005). This was seen at T4, T5, and T6, which represented a period where the number of matches played increased. The majority of the available research reports a decrease in sIgA being observed after a soccer match (Fredericks, Fitzgerald, Shaw & Holt, 2012; Moreira, Arsati, de Oliveira Lima-Arsati, de Freitas & de Araujo, 2011). However it has been reported that an increase in sIgA secretion occurs in response to intense soccer specific exercise (Sari-Sarraf, Reilly, Doran & Atkinson, 2007). The significant increase at T8 was also coupled with an increase in CK and a decrease in cortisol when compared T6. Attributing a specific factor as to why a sharp increase at T8 alone occurred is challenging. Intriguingly this time point coincided with the change of manager for the team. Additionally, various authors have reported the increase in risk of URTI with suppression in sIgA concentrations (Neville, Gleeson & Folland, 2008; Orysiak, Malczewska-Lenczowska, Szyguła & Pokrywka, 2012). Periodising nutritional strategies to optimise immune function is an area that could lead

to increased player availability (Phillips & Van Loon, 2011). Using this data could also provide direction as to when to incorporate structured recovery sessions into the training program. Moreira, Mortatti, Arruda, Freitas, de Arruda and Aoki (2014) reported that a 2-week prophylactic recovery period induced a significant increase in sIgA secretion rate and a decrease in URTI symptoms ($p < 0.05$). Owen et al. (2014) observed the last period of training over a week showed the greatest decrease in sIgA concentrations ($p < 0.05$), suggesting that repeated exposure to high intensity training loads could lead to increased fatigue and immune suppression. Knowing when in the season this suppression of sIgA occurs is valuable to plan the inclusion of recovery and lower intensity training sessions. Finally, at T9 there was a decrease in sIgA concentrations, which was coupled with a significant increase in cortisol ($p = 0.0001$). Previous research has reported an inverse relationship between sIgA and cortisol (Clements, 2013; He, Tsai, Ko, Chang and Fang, 2010). Analysing figure 5 it can be seen that this relationship occurs throughout the season with increases in sIgA being coupled with suppression of cortisol levels. At T2 sIgA levels rose from 119.1 to 194.3 $\mu g.mL^{-1}$ whilst cortisol levels decreased from 6.53 to 5.42 ng/ml. This relationship continued during T4, T5, T6 and T7. Increasing the frequency of sampling could provide more detailed information as to the responses to matches and training.

Cortisol

The principal finding in this study was a significant increase in cortisol levels at both T8 ($p = 0.03$) and T9 ($p = 0.0001$) compared to T1. Numerous studies

have reported that cortisol has a pivotal role in the physiological response to a physical challenge or to a psychological stressor (Clements, 2013; Thorpe & Sunderland, 2012), with cortisol tending to heighten its response to an increase in volume or stress (Brownlee, Moore & Hackney, 2005). Investigations into the behaviour of cortisol over the course of a soccer season have also suggested that that the final 9 weeks of the season cause an increase in concentrations (Meister, Faude, Ammann, Schnittker & Meyer, 2013). Both T8 and T9 represented matches towards the end of the season, which coincided with a change of manager that could of contributed to the spike in cortisol levels. Haneishi et al. (2007) noted that competition induced greater hormonal responses in collegiate soccer players when compared to a regular training session. This is supported by investigations suggesting that psychological element of competition can have a sizeable influence on cortisol response (Doan, Newton, Kraemer, Kwon, & Scheet, 2007). Elloumi et al. (2003) reported that in 20 elite rugby players, cortisol levels were significantly raised (148%, $p < 0.05$) after a rugby match, but when the competition element was removed and they simulated similar physiological activity in the laboratory, cortisol levels did not respond to the same magnitude. The reduction in cortisol levels from competition to mirrored laboratory exercise also described in elite handball and volleyball players (Filaire et al. 1999). The observation that three of the biomarkers tested displayed significant variations at T8 and T9 suggests that the psychological component could be a noteworthy factor. Furthermore, the influence of accumulative fatigue could be a reason for the increase in cortisol levels at T8 and T9 (Da Silva, Papoti, Santhiago, Pauli & Gobatto, 2011). An increase in

cortisol has been reported to have a negative impact on anaerobic performance. Ispirildis et al. (2008) along with West et al. (2014) observed a negative correlation between cortisol and neuromuscular output with a reduction in power output occurring up to 60 hours post match in elite soccer and rugby players respectively. The results from the current study are most in line with that of Kraemer et al. (2004), who investigated the changes in hormonal concentrations in 25 college soccer players over a season. There were no significant changes in plasma cortisol concentrations during the course of the season, with the exception of time point 4 (T4) where a significant increase in cortisol concentrations was observed ($p < 0.05$). Coincidentally T4 was the penultimate time point measured in the season, similarly to that of T7 in the current study.

Urea

In addition to the significant increases at T8 and T9 ($p = 0.04$; $p = 0.01$), a small steady increase over the season was observed in mean urea scores (7.82 ± 1.1 mmol/L to 9.77 ± 1.6 mmol/L). These findings are in agreement with that of Meister, Faude, Ammann, Schnittker and Meyer (2013) and Silva, Santhiago, Papoti and Gobatto (2008) who both reported no change in urea scores in elite soccer players, urea is regarded as a marker of high physical strain (Meyer & Meister, 2011). However, Andersson, Raastad, Nilsson, Paulsen, Garthe and Kadi (2010) did observe a significant increase ($p < 0.05$) in urea after two competitive matches in elite female soccer players. The contrast in these findings could be attributed to the close proximity of the matches in the Anderson et al. (2008) study (two matches in four days)

whereas the current study sampled players at the end of each month.

The observation of a significant high positive correlation (r = 0.76; p < 0.05) between urea and cortisol over the season is a supported by one other study in elite soccer (Filaire, Lac & Pequignot, 2003). The authors also observed a significant increase in both urea and cortisol over the course of a season in elite French soccer players. Increased cortisol levels can disrupt homeostasis and with concurrent increases in urea, targeted nutritionally strategies may potentially offer a method to reduce both biomarkers. Phillips and Van Loon (2011) have suggested a higher protein and carbohydrate intake is required in elite athletes. Increasing protein intake may aid in the controlling the rise in urea as it prevents protein breakdown and provides adequate amino acids for muscle recovery and growth. Carbohydrate has been shown to reduce cortisol levels when taken post exercise (Lane, Duke & Hackney, 2010) with a combined protein/carbohydrate meal or shake being suggested as the optimal nutritional strategy post exercise (Williams, 2005).

Creatine Kinase

In this study no significant change was observed in CK levels over the course of the season. As the first time point was taken after the 2nd competitive match, some training induced adaptations of blood parameters may have occurred. These changes mainly consist of an increase in plasma volume and subsequent total haemoglobin that can lead to an increase in endurance capacity (Brun, 2002). The highest CK levels recorded were at T7, which interestingly preceded a significant increase in sIgA and cortisol at T8 (p < 0.05). It has been reported that elite athletes have adapted to respond less

pronounced increases in CK than sedentary subjects, but due to the high frequency of matches and inadequate recovery in premier league soccer, some researchers have stated that soccer players can have consistently elevated CK levels (Heisternerg, Fahrenkrug, Krustrup, Storskov, Kjær & Andersen, 2013). In the study by Heisterberg et al. (2013) 54% of the CK samples were above 270 UI/L, which has been set as the upper reference value for the general population (Mougios, 2007). Overall the changes in CK levels throughout the season were not variable enough to justify as any signs of over-reaching or fatigue (Lazarim et al. 2009). Lastly, the data supports the hypothesis that CK levels are extremely individual with large differences being observed (57 IU/L – 625 IU/L). Research conducted on elite soccer players has also reported large variability in CK levels, even with similar training loads (Meister, Faude, Ammann, Schnittker & Meyer, 2013).

Limitations to the study

The absence of a baseline measurement for all biomarkers is a factor that makes analysing the data challenging. Using T1 as the first time point meant that training adaptation, psychological and physiological factors associated with competitive match play could have influenced the sample data. Furthermore the lack of physical load data from the matches lead to the speculation as to the causes of the variations observed. Future studies should look to perform a routine screening at the beginning of the season to establish baseline values, with a view then to analyse the biomarkers against training and match physical load data. This will allow for a quantifiable analysis of why specific variations may have occurred. However, this study reflects real

competitive match exposure and thus, has high ecological validity.

Conclusion

An increase in the catabolic environment that occurs during the end of the season may have contributed to the significant variations in the specific biomarkers. Fatigue, increased physiological stress leading to an increase in protein breakdown, increased stress from the importance of the matches alongside the change in manager may have contributed to the variations noted in this study. This study has indicated that the differing time periods during the season can result in significant changes in players' physiological status. With the continued high intensity stress experienced over the course of the season, the consequence of entering the end the season period in a sub optimal physiological state could lead to a reduction in performance. When load becomes overload it can lead to under performance, immune suppression and impaired recovery. The conclusions made could provide valuable information to staff and coaches to enable them to plan the training load based on physiological responses to match play. Towards the end of the season when the matches are of higher importance, this insight could be crucial.

1224529

References

Andersson, H., Bøhn, S. K., Raastad, T., Paulsen, G., Blomhoff, R., & Kadi, F. (2010). Differences in the inflammatory plasma cytokine response following two elite female soccer games separated by a 72-h recovery. *Scandinavian Journal of Medicine and Science In Sports*, *20*(5), 740-747.

Ascensão, A., Rebelo, A., Oliveira, E., Marques, F., Pereira, L., & Magalhães, J. (2008). Biochemical impact of a soccer match—analysis of oxidative stress and muscle damage markers throughout recovery. *Clinical Biochemistry*, *41*(10), 841-851.

Baird, M. F., Graham, S. M., Baker, J. S., & Bickerstaff, G. F. (2012). Creatine-kinase-and exercise-related muscle damage implications for muscle performance and recovery. *Journal of Nutrition and Metabolism*, 960363-960363.

Bloomfield, J., Polman, R., & O'Donoghue, P. (2007). Physical demands of different positions in FA Premier League soccer. *Journal of Sports Science and Medicine*, *6*(1), 63.

Bradley, P. S., Sheldon, W., Wooster, B., Olsen, P., Boanas, P., & Krustrup, P. (2009). High-intensity running in English FA Premier League soccer matches. *Journal of Sports Sciences*, *27*(2), 159-168.

Brancaccio, P., Limongelli, F. M., & Maffulli, N. (2006). Monitoring of serum enzymes in sport. *British Journal of Sports Medicine*, *40*(2), 96-97.

1224529

Brancaccio, P., Maffulli, N., & Limongelli, F. M. (2007). Creatine kinase monitoring in sport medicine. *British Medical Bulletin*, *81*(1), 209-230.

Brownlee, K. K., Moore, A. W., & Hackney, A. C. (2005). Relationship between circulating cortisol and testosterone: influence of physical exercise. *Journal of Sports Science and Medicine*, *4*(1), 76.

Brun, J, (2002). Exercise hemorheology as a three acts play with metabolic actors: Is it of clinical relevance? *Clinical Hemorheology Microcirculation*, 26(3), 155-174

Clements, A. D. (2013). Salivary cortisol measurement in developmental research: Where do we go from here? *Developmental Psychobiology*, *55*(3), 205-220.

Da Silva, A. S. R., Papoti, M., Santhiago, V., Pauli, J. R., & Gobatto, C. A. (2011). Serum and plasma hormonal concentrations are sensitive to periods of intensity and volume of soccer training. *Science and Sports*, *26*(5), 278-285.

Dellal, A., Chamari, K., Wong, D. P., Ahmaidi, S., Keller, D., Barros, R., ... & Carling, C. (2011). Comparison of physical and technical performance in European soccer match-play: FA Premier League and La Liga. *European Journal of Sport Science*, *11*(1), 51-59.

Doan, B. K., Newton, R. U., Kraemer, W. J., Kwon, Y. H., & Scheet, T. P. (2007). Salivary cortisol, testosterone, and T/C ratio responses during a 36-hole golf competition. *International Journal of Sports Medicine*, *28*(6), 470-479.

1224529

Dunbar, J., Jehanli, A., & Browne, A. (2014). Investigating The Use of a Point Of Care Salivary IgG Test In The Sporting Environment. *International Journal of Exercise Science: Conference Proceedings* (Vol. 10, No. 1, p. 57).

Elloumi, M., Maso, F., Michaux, O., Robert, A., & Lac, G. (2003). Behaviour of saliva cortisol [C], testosterone [T] and the T/C ratio during a rugby match and during the post-competition recovery days. *European Journal of Applied Physiology*, *90*(1-2), 23-28.

Fatouros, I. G., Chatzinikolaou, A., Douroudos, I. I., Nikolaidis, M. G., Kyparos, A., Margonis, K., ... & Jamurtas, A. Z. (2010). Time-course of changes in oxidative stress and antioxidant status responses following a soccer game. *The Journal of Strength and Conditioning Research*, *24*(12), 3278-3286.

Filaire, E., Scanff, C. L., Duche, P., & Lac, G. (1999). The relationship between salivary adrenocortical hormones changes and personality in elite female athletes during handball and volleyball competition. *Research quarterly for exercise and sport*, *70*(3), 297-302.

Filaire, E., Bernain, X., Sagnol, M., & Lac, G. (2001). Preliminary results on mood state, salivary testosterone: cortisol ratio and team performance in a professional soccer team. *European Journal of Applied Physiology*, *86*(2), 179-184.

1224529

Filaire, E., Lac, G., & Pequignot, J. M. (2003). Biological, hormonal, and psychological parameters in professional soccer players throughout a competitive season. *Perceptual and Motor Skills*, *97*(3 Pt 2), 1061.

Fragala, M. S., Kraemer, W. J., Denegar, C. R., Maresh, C. M., Mastro, A. M., & Volek, J. S. (2011). Neuroendocrine-immune interactions and responses to exercise. *Sports Medicine*, *41*(8), 621-639.

Fredericks, S., Fitzgerald, L., Shaw, G., & Holt, D. W. (2012). Changes in Salivary Immunoglobulin A (IgA) following Match-play and Training among English Premiership Footballers. *Medicine Journal of Malaysia*, *67*(2), 155.

Gleeson, M., & Walsh, N. P. (2012). The BASES expert statement on exercise, immunity, and infection. *Journal of Sports Sciences*, *30*(3), 321-324.

Gleeson, M., Pyne, D. B., & Callister, R. (2003). Exercise effects on mucosal immunity and risk of upper respiratory illness: review article. *International Sports Medicine Journal*, *4*(3), 1-14.

Gozansky, W. S., Lynn, J. S., Laudenslager, M. L., & Kohrt, W. M. (2005). Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic–pituitary–adrenal axis activity. *Clinical Endocrinology*, *63*(3), 336-341.

Haneishi, K., Fry, A. C., MOORE, C. A., Schilling, B. K., Li, Y., & Fry, M. D. (2007). Cortisol and stress responses during a game and practice in female collegiate

soccer players. *The Journal of Strength and Conditioning Research*, *21*(2), 583-588.

He, C. S., Tsai, M. L., Ko, M. H., Chang, C. K., & Fang, S. H. (2010). Relationships among salivary immunoglobulin A, lactoferrin and cortisol in basketball players during a basketball season. *European Journal of Applied Physiology*, *110*(5), 989-995.

Heisterberg, M. F., Fahrenkrug, J., Krustrup, P., Storskov, A., Kjær, M., & Andersen, J. L. (2013). Extensive monitoring through multiple blood samples in professional soccer players. *The Journal of Strength and Conditioning Research*, *27*(5), 1260-1271.

Hellhammer, D. H., Wüst, S., & Kudielka, B. M. (2009). Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology*, *34*(2), 163-171.

Hucklebridge, F., Clow, A., & Evans, P. (1998). The relationship between salivary secretory immunoglobulin A and cortisol: neuroendocrine response to awakening and the diurnal cycle. *International Journal of Psychophysiology*, *31*(1), 69-76.

Hunkin, S. L., Fahrner, B., & Gastin, P. B. (2014). Creatine kinase and its relationship with match performance in elite Australian Rules football. *Journal of Science and Medicine in Sport*, *17*(3), 332-336.

Ispirlidis, I., Fatouros, I. G., Jamurtas, A. Z., Nikolaidis, M. G., Michailidis, I., Douroudos, I., ... & Taxildaris, K. (2008). Time-course of changes in inflammatory and performance responses following a soccer game. *Clinical Journal of Sport Medicine*, *18*(5), 423-431.

Kraemer, W. J., & Ratamess, N. A. (2005). Hormonal responses and adaptations to resistance exercise and training. *Sports Medicine*, *35*(4), 339-361.

Kraemer, W. J., French, D. N., Paxton, N. J., Häkkinen, K., Volek, J. S., Sebastianelli, W. J., ... & Knuttgen, H. G. (2004). Changes in exercise performance and hormonal concentrations over a big ten soccer season in starters and nonstarters. *The Journal of Strength and Conditioning Research*, *18*(1), 121-128.

Lane, A. R., Duke, J. W., & Hackney, A. C. (2010). Influence of dietary carbohydrate intake on the free testosterone: cortisol ratio responses to short-term intensive exercise training. *European Journal of Applied Physiology*, *108*(6), 1125-1131.

Lazarim, F. L., Antunes-Neto, J. M., da Silva, F. O., Nunes, L. A., Bassini-Cameron, A., Cameron, L. C., ... & de Macedo, D. V. (2009). The upper values of plasma creatine kinase of professional soccer players during the Brazilian National Championship. *Journal of Science and Medicine in Sport*, *12*(1), 85-90.

Malm, C, Ekblom, O, and Ekblom, B. (2004). Immune system alteration in response to two consecutive soccer games. *Acta Physiologica Scandinavica*, *180*(2), 143-155.

Meister, S., aus der Fünten, K., & Meyer, T. (2014). Repeated monitoring of blood parameters for evaluating strain and overload in elite football players: is it justified? *Journal of Sports Sciences*, *32*(13), 1328-1331.

Meister, S., Faude, O., Ammann, T., Schnittker, R., & Meyer, T. (2013). Indicators for high physical strain and overload in elite football players. *Scandinavian Journal of Medicine and Science In Sports*, *23*(2), 156-163.

Meyer, T., & Meister, S. (2011). Routine blood parameters in elite soccer players. *International Journal of Sports Medicine*, *32*(11), 875-881.

Moreira, A., Arsati, F., de Oliveira Lima-Arsati, Y. B., de Freitas, C. G., & de Araujo, V. C. (2011). Salivary immunoglobulin A responses in professional top-level futsal players. *The Journal of Strength and Conditioning Research*, *25*(7), 1932-1936.

Moreira, A., Mortatti, A. L., Arruda, A. F., Freitas, C. G., de Arruda, M., & Aoki, M. S. (2014). Salivary IgA Response and Upper Respiratory Tract Infection Symptoms During a 21-Week Competitive Season in Young Soccer Players.*The Journal of Strength and Conditioning Research*, *28*(2), 467-473.

Mougios, V. (2007). Reference intervals for serum creatine kinase in athletes.*British Journal of Sports Medicine*, *41*(10), 674-678.

Neville, V., Gleeson, M., & Folland, J. P. (2008). Salivary IgA as a risk factor for upper respiratory infections in elite professional athletes. *Medicine and Science In Sports and Exercise*, *40*(7), 1228-1236.

Orysiak, J., Malczewska-Lenczowska, J., Szyguła, Z., & Pokrywka, A. (2012). The role of salivary immunoglobulin A in the prevention of the upper respiratory tract infections in athletes–an overview. *Biology of Sport*, *29*(4), 311.

1224529

Owen, L., Wong, D. P., Dunlop, G., Groussard, C., Kebsi, W., Dellal, A., ... & Cachan, F. (2014). High Intensity Training and Salivary Immunoglobulin-A Responses in Professional Top-Level Soccer Players: Effect of Training Intensity. *Journal of Strength and Conditioning Research,* [Epub ahead of print].

Papacosta, E., & Nassis, G. P. (2011). Saliva as a tool for monitoring steroid, peptide and immune markers in sport and exercise science. *Journal of Science and Medicine in Sport*, *14*(5), 424-434.

Papacosta, E., Gleeson, M., & Nassis, G. P. (2013). Salivary Hormones, IgA, and Performance During Intense Training and Tapering in Judo Athletes. *The Journal of Strength and Conditioning Research*, *27*(9), 2569-2580.

Phillips, S. M., & Van Loon, L. J. (2011). Dietary protein for athletes: from requirements to optimum adaptation. *Journal of Sports Sciences*, *29*(sup1), S29-S38.

Sari-Sarraf, V., Reilly, T., Doran, D. A., & Atkinson, G. (2007). The effects of single and repeated bouts of soccer-specific exercise on salivary IgA. *Archives of Oral Biology*, *52*(6), 526-532.

Silva, A. S. R., Santhiago, V., Papoti, M., & Gobatto, C. A. (2008). Psychological, biochemical and physiological responses of Brazilian soccer players during a training program. *Science and Sports*, *23*(2), 66-72.

Silva, J. R., Rebelo, A., Marques, F., Pereira, L., Seabra, A., Ascensão, A., & Magalhães, J. (2013). Biochemical impact of soccer: an analysis of hormonal, muscle damage, and redox markers during the season. *Applied Physiology, Nutrition and Metabolism*, *39*(4), 432-438.

Thorpe, R., & Sunderland, C. (2012). Muscle damage, endocrine, and immune marker response to a soccer match. *The Journal of Strength and Conditioning Research*, *26*(10), 2783-2790.

Tiollier, E., Gomez-Merino, D., Burnat, P., Jouanin, J. C., Bourrilhon, C., Filaire, E., ... & Chennaoui, M. (2005). Intense training: mucosal immunity and incidence of respiratory infections. *European Journal of Applied Physiology*, *93*(4), 421-428.

Viru, A. A., & Viru, M. (2001). *Biochemical Monitoring Of Sport Training*. Human Kinetics.

Walsh, N., Laing, S., Oliver, S., Montague, J., Walters, R. & Bilzon, J. (2004). Saliva parameters as potential indices of hydration status during acute dehydration. *Medicine and Science in Sports and Exercise*, *36*(9), 1535-1542

West, D. J., Finn, C. V., Cunningham, D. J., Shearer, D. A., Jones, M. R., Harrington, B. J., ... & Kilduff, L. P. (2014). Neuromuscular Function, Hormonal, and Mood Responses to a Professional Rugby Union Match. *The Journal of Strength and Conditioning Research*, *28*(1), 194-200.

Williams, J. H. H. (2005). The effects of a high carbohydrate diet on cortisol and salivary immunoglobulin A (s-IgA) during a period of increase exercise

workload amongst Olympic and Ironman triathletes. *International Journal of Sports Medicine*, *26*, 880-885.

Faculty of Life Sciences

Research Ethics Committee

frec@chester.ac.uk

Liam Holmes

19th June 2014

Dear Liam,

Study title: **Examining the biochemical and physiological responses in elite professional soccer players throughout a competitive season.**

FREC reference: **947/14/LH/CSN**

Version number: **1**

Thank you for sending your application to the Faculty of Life Sciences Research Ethics Committee for review.

I am pleased to confirm ethical approval for the above research, provided that you comply with the conditions set out in the attached document, and adhere to the processes described in your application form and supporting documentation.

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date

Application Form	1	May 2014
Appendix 1 – List of References	1	May 2014
Appendix 2 – C.V. for Lead Researcher	1	May 2014
Appendix 3 – Written Permission, Performance Director, Fulham Football Club	1	May 2014

Please note that this approval is given in accordance with the requirements of English law only. For research taking place wholly or partly within other jurisdictions (including Wales, Scotland and Northern Ireland), you should seek further advice from the Committee Chair / Secretary or the Research and Knowledge Transfer Office and may need additional approval from the appropriate agencies in the country (or countries) in which the research will take place.

With the Committee's best wishes for the success of this project.

Yours sincerely,

Dr. Stephen Fallows

Chair, Faculty Research Ethics Committee

Enclosures: Standard conditions of approval.

Cc. Supervisor/FREC Representative